

### **REMARKS/ARGUMENTS**

Upon entry of this amendment, claims 18-54, 56-59, 62, and 67 are pending in this application and are presented for examination. Claims 47, 52-54, 56, 58, and 62 have been amended. Claims 55, 60, 61, and 63-66 have been canceled without prejudice. Claim 67 has been newly added. Claims 18-46 have been withdrawn from consideration as being drawn to a non-elected invention pursuant to a Restriction Requirement. No new matter has been introduced with the foregoing amendment. Reconsideration is respectfully requested.

#### **I. FORMALITIES**

Claim 47 has been amended to recite that the method comprises increasing the expression or activity of an ER resident calcium-binding protein in the cell by introducing a polynucleotide operably linked to a promoter into the cell, wherein the polynucleotide encodes the ER resident calcium-binding protein. Support for amended claim 47 is found, for example, on page 8, lines 8-11, on page 16, lines 3-23, and on page 22, lines 22-26.

Claim 52 has been amended to replace the term "ER resident chaperone protein" with "ER resident calcium-binding protein." Support for amended claim 52 is found, for example, on page 16, lines 16-23.

Claim 53 has been amended to replace the term "ER resident chaperone protein" with "ER resident calcium-binding protein" and to add reticulocalbin to the list of ER resident calcium-binding proteins. Support for amended claim 53 is found, for example, on page 8, lines 8-11, on page 16, lines 12-13, and on page 16, lines 16-23.

Claim 54 has been amended to replace the term "production" with "increase in the expression or activity" and to replace the term "ER resident chaperone protein" with "ER resident calcium-binding protein." Support for amended claim 54 is found, for example, on page 16, lines 3-23.

Claims 56 and 58 have been amended to properly depend from claim 47.

Claim 62 has been amended to recite that the method comprises increasing the expression or activity of an ER resident calcium-binding protein in the cell by administering a

proinflammatory cytokine to the cell. New claim 67 recites that the proinflammatory cytokine is interleukin-3 (IL-3). Support for amended claim 62 and new claim 67 is found, for example, on page 17, lines 1-4. The reference cited therein, Brewer *et al.*, *EMBO J.*, 16:7207-7216 (1997), discloses that IL-3 and colony stimulating factor-1 (CSF-1) induce the expression of ER resident calcium-binding proteins. Applicants submit that prior to the filing of the instant application, it was well known in the art that both IL-3 and CSF-1 are proinflammatory cytokines (*see, e.g.*, Yun *et al.*, *Life Sci.*, 67:2855-2863 (2000); abstract). As such, Applicants believe that the term "proinflammatory cytokine" is supported in the specification as filed.

Thus, no new matter has been introduced. As such, Applicants respectfully request that the amended claims and new claim be entered.

## **II. OBJECTION TO THE DRAWINGS**

As indicated in the Amendment dated August 27, 2003, Applicants will file formal drawings upon receiving a Notice of Allowance.

## **III. REJECTION UNDER 35 U.S.C. § 112, FIRST PARAGRAPH**

Claims 47-66 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with the claims. To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection.

The Examiner alleges that the specification, while being enabling for a method for inhibiting the generation of active thrombin on the surface of a cell within a mammal wherein the method comprises: (1) directly administering to the cell a polynucleotide which encodes and expresses GRP78/BiP, or (2) administering to the cell interleukin-3 or interleukin-10, does not reasonably provide enablement for the full scope encompassed by the claims.

In support of the above rejection, the Examiner raises the following two enablement concerns: (1) the claims encompass administering any compound that activates or increases the level or expression of any ER resident chaperone protein; and (2) the claims

encompass administering a nucleic acid that encodes and expresses any ER resident chaperone protein to the target cell by any means of administration such as systemic administration.

In order to expedite prosecution of the present case, Applicants have amended claim 47 to recite a method of inhibiting the generation of active thrombin on the surface of a cell within an atherosclerotic plaque within a mammal comprising increasing the expression or activity of an ER resident calcium-binding protein in the cell by introducing a polynucleotide operably linked to a promoter into the cell, wherein the ***polynucleotide encodes the ER resident calcium-binding protein***. As presently amended, claim 47 does not encompass administering any compound that activates or increases the level or expression of any ER resident chaperone protein, but instead encompasses the introduction of a polynucleotide encoding a specific class of ER resident chaperone protein, *i.e.*, an ER resident calcium-binding protein, into the cell. Applicants submit that the application, at the time of filing, contained sufficient information so as to enable one of skill in the art to make and use the claimed invention without undue experimentation. In particular, the instant specification teaches that increasing the expression or activity of ER resident calcium-binding proteins such as GRP78/BiP, GRP94, reticulocalbin, calreticulin, and calnexin can be used to inhibit the generation of active thrombin on the surface of a cell (*see*, page 8, lines 3-11 and page 16, lines 3-23). As such, it is readily apparent that the instant specification enables the full scope of amended claim 47 and, thus, undue experimentation is ***not*** required to practice the full scope of the claim. In view of the foregoing, Applicants respectfully request that the Examiner withdraw the 35 U.S.C. § 112, first paragraph rejection.

The Examiner also alleges that because Dai *et al.*, *Arteriosclerosis, Thrombosis, and Vascular Biology*, 17:2359-2368 (1997) ("Dai *et al.*") disclose that the calcium-binding protein calsequestrin does not decrease plaque area, "additional experimentation is required in order to determine which calcium decreasing molecules would work in the claimed methods and which would not work." In response, Applicants respectfully point out that calsequestrin is ***not*** an ER resident calcium-binding protein. Rather, calsequestrin is a calcium-binding protein residing in the sarcoplasmic reticulum (SR) that is responsible for maintaining the level of

intracellular calcium in cardiac and skeletal muscle by storing and releasing calcium. As the ER and SR are clearly distinct intracellular compartments performing distinct functions, *e.g.*, the ER being involved in the secretory pathway and the SR being involved in muscle contractility, Applicants assert that calsequestrin is not within the scope of amended claim 47, which recites that the polynucleotide encodes an ***ER resident*** calcium-binding protein. As such, additional experimentation would *not* be required to practice the full scope of the claim. In view of the foregoing, Applicants respectfully request that the Examiner withdraw the 35 U.S.C. § 112, first paragraph rejection.

In addition, the Examiner alleges that "it is unpredictable that the nucleic acid of interest could be administered systemically to a mammal and result in the specific transfection of the target cells." In response, Applicants respectfully point out that the instant specification teaches the use of liposomal formulations for introducing polynucleotides into cells (*see*, page 19, line 18 to page 21, line 3), and that liposomal formulations for delivering nucleic acids systemically were known to those of skill in the art at the time of the present invention (*see, e.g.*, PCT Publication No. 99/39741). In particular, the instant specification teaches the use of immunoliposomes as carriers of exogenous polynucleotides that "may have improved cell type specificity as compared to liposomes by virtue of the inclusion of specific antibodies which presumably bind to surface antigens on specific cell types" (page 19, lines 30-34). Applicants submit that immunoliposomes were being successfully and predictably used to systemically deliver exogenous polynucleotides to target cells at the time of the present invention. For example, Wang *et al.*, *PNAS*, 84: 7851-7855 (1987) disclose the use of immunoliposomes for intraperitoneally delivering exogenous genes specifically to lymphoma cells, as nonspecific delivery to tissues such as the spleen was significantly reduced (*see*, attached abstract). Further, Park *et al.*, *Cancer Lett.*, 118:153-160 (1997) disclose the use of immunoliposomes for intravenously delivering doxorubicin specifically to target tumor cells and teach that such immunoliposomes can also mediate efficient and specific transfection of target tumor cells with exogenous genes (*see*, attached abstract). Moreover, Shi *et al.*, *PNAS*, 97:7567-7572 (2000) disclose the use of immunoliposomes for intravenously delivering exogenous genes specifically

to the central nervous system (*see*, attached abstract). Such immunoliposomes were stable in blood and were not selectively entrapped in the lung. As such, Applicants assert that the teachings of the instant specification, coupled with the general knowledge in the art at the time of the present invention, provide modes of delivery such as immunoliposomes that allow for systemic administration of nucleic acids to target cells. Thus, undue experimentation would *not* be needed to carry out the methods of the present invention. In view of the foregoing, Applicants respectfully request that the Examiner withdraw the 35 U.S.C. § 112, first paragraph rejection.

#### IV. REJECTION UNDER 35 U.S.C. § 102(b): *Hansson et al.*

Claims 47-54, 60, and 61 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by U.S. Patent No. 5,208,019 ("*Hansson et al.*"). In particular, the Examiner alleges that the claims "encompass any method for producing an ER chaperone protein in an atherosclerotic plaque cell, including administering a cytokine, such as gamma-interferon, to target cells." To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection.

As discussed above, in order to expedite prosecution, Applicants have amended claim 47 to recite a method of inhibiting the generation of active thrombin on the surface of a cell within an atherosclerotic plaque within a mammal comprising increasing the expression or activity of an ER resident calcium-binding protein in the cell by introducing a polynucleotide operably linked to a promoter into the cell, wherein the polynucleotide encodes the ER resident calcium-binding protein. In contrast, *Hansson et al.* only disclose the use of ***recombinant gamma-interferon protein*** to inhibit the growth or proliferation of arterial smooth muscle cells. As a result, Applicants assert that *Hansson et al.* in no way teach or suggest the introduction of a ***polynucleotide encoding an ER resident calcium-binding protein*** into a cell to inhibit the generation of active thrombin on the surface of the cell within an atherosclerotic plaque.

As such, each and every element as set forth in amended claim 47 is not found in *Hansson et al.*, and the method claimed in amended claim 47 would therefore not be anticipated

by the teachings of Hansson *et al.* In addition, all claims that depend from claim 47, including claims 48-54, would not be anticipated by the teachings of Hansson *et al.* Claims 60 and 61 have been canceled without prejudice, thereby rendering the rejection moot for these claims. In view of the foregoing, Applicants respectfully request that the Examiner withdraw the 35 U.S.C. § 102(b) rejection.

**V. REJECTION UNDER 35 U.S.C. § 102(b): Dai *et al.***

Claims 47-51, 53, and 54 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Dai *et al.* In particular, the Examiner alleges that the "administration of calreticulin to the site of plaque development (as taught by Dai) meets all of [the] limitations of the claim required to result in 'producing an ER resident chaperone protein (and specifically, calreticulin) in a cell within an atherosclerotic plaque in an animal.'" To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection.

As discussed above, in order to expedite prosecution, Applicants have amended claim 47 to recite a method of inhibiting the generation of active thrombin on the surface of a cell within an atherosclerotic plaque within a mammal comprising increasing the expression or activity of an ER resident calcium-binding protein in the cell by introducing a polynucleotide operably linked to a promoter into the cell, wherein the polynucleotide encodes the ER resident calcium-binding protein. In contrast, Dai *et al.* only disclose the use of ***calreticulin protein*** to reduce intimal hyperplasia following arterial injury. Further, Dai *et al.* disclose in the last paragraph of the Discussion that "the antiatherosclerotic activity of calreticulin is the result of the multifunctional nature of calreticulin, which allows calreticulin to exert local effects at sites of endothelial injury ***through binding to the cell surface***" (emphasis added). As a result, Applicants assert that Dai *et al.* in no way teach or suggest the introduction of a ***polynucleotide*** encoding an ER resident calcium-binding protein ***into a cell*** to inhibit the generation of active thrombin on the surface of the cell within an atherosclerotic plaque.

As such, each and every element as set forth in amended claim 47 is not found in Dai *et al.*, and the method claimed in amended claim 47 would therefore not be anticipated by

the teachings of Dai *et al.* In addition, all claims that depend from claim 47, including claims 48-51, 53, and 54, would not be anticipated by the teachings of Dai *et al.* In view of the foregoing, Applicants respectfully request that the Examiner withdraw the 35 U.S.C. § 102(b) rejection.

**VI. REJECTION UNDER 35 U.S.C. § 102(a): Mallat *et al.***

Claims 47-54, 60, and 61 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Mallat *et al.*, *Circulation Research*, 85:e17-e24 (1999) ("Mallat *et al.*"). In particular, the Examiner alleges that the claims "encompass any method for producing an ER chaperone protein in an atherosclerotic plaque cell, including administering a cytokine such as IL-10 to the target cells." To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection.

As discussed above, in order to expedite prosecution, Applicants have amended claim 47 to recite a method of inhibiting the generation of active thrombin on the surface of a cell within an atherosclerotic plaque within a mammal comprising increasing the expression or activity of an ER resident calcium-binding protein in the cell by introducing a polynucleotide operably linked to a promoter into the cell, wherein the polynucleotide encodes the ER resident calcium-binding protein. In contrast, Mallat *et al.* only disclose the use of a ***polynucleotide encoding the anti-inflammatory cytokine IL-10*** to reduce fatty lesion development. Further, Mallat *et al.* disclose that the reduction in fatty lesion development was due to "markedly increased ***peripheral circulating levels of IL-10***" (*see*, right column, bottom paragraph on page e23; emphasis added). As a result, Applicants assert that Mallat *et al.* in no way teach or suggest the introduction of a ***polynucleotide encoding an ER resident calcium-binding protein into a cell*** to inhibit the generation of active thrombin on the surface of the cell within an atherosclerotic plaque.

As such, each and every element as set forth in amended claim 47 is not found in Mallat *et al.*, and the method claimed in amended claim 47 would therefore not be anticipated by the teachings of Mallat *et al.* In addition, all claims that depend from claim 47, including claims

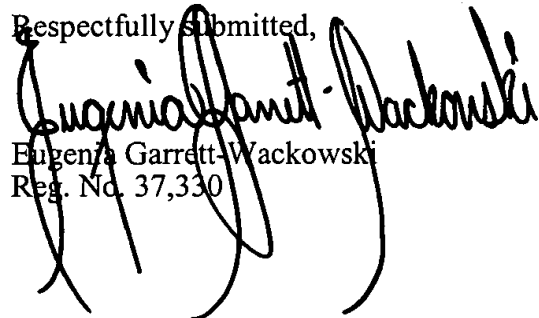
48-54, would not be anticipated by the teachings of Mallat *et al.* Claims 60 and 61 have been canceled without prejudice, thereby rendering the rejection moot for these claims. In view of the foregoing, Applicants respectfully request that the Examiner withdraw the 35 U.S.C. § 102(b) rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

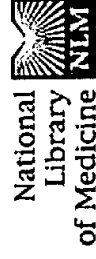
Respectfully submitted,



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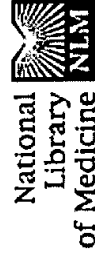
## pH-sensitive immunoliposomes mediate target-cell-specific delivery and controlled expression of a foreign gene in mouse.

Wang CY, Huang L.

Department of Biochemistry, University of Tennessee, Knoxville 37996-0840.

A plasmid containing the Escherichia coli chloramphenicol acetyltransferase (CAT) gene under the control of a mammalian cAMP-regulated promoter was entrapped in H-2Kk antibody-coated liposomes composed of dioleoyl phosphatidylethanolamine, cholesterol, and oleic acid (pH-sensitive immunoliposomes). The entrapped or free DNA was injected intraperitoneally into immunodeficient (nude) BALB/c mice bearing ascites tumor generated by H-2Kk-positive RDM-4 lymphoma cells. About 20% of the injected immunoliposomes were taken up by the target RDM-4 cells. Uptake was much less when liposomes without antibody were used. The presence of the targeting antibody on liposomes also significantly decreased the nonspecific uptake of liposomes by the spleen. Significant CAT enzyme activity was detected in RDM-4 cells from mice treated with DNA entrapped in the pH-sensitive immunoliposomes. Furthermore, CAT expression in RDM-4 cells was under the control of cAMP, as only the cells from mice injected with 8-bromo-cAMP and 3-isobutyl-1-methylxanthine showed CAT activity. CAT activity in liver and spleen was much lower (by factors of 12 and 5, respectively) than in the RDM-4 cells, and the activities in these reticuloendothelial organs were not regulated by cAMP. CAT activity in RDM-4 cells from mice injected with DNA entrapped in pH-insensitive immunoliposomes (containing phosphatidylcholine in place of phosphatidylethanolamine) was approximately one-fourth that in RDM-4 cells from mice injected with pH-sensitive immunoliposomes, indicating the superior delivery efficiency of the pH-sensitive liposomes. These results are discussed in terms of the DNA-carrier potential of immunoliposomes in therapy of cancer and genetic diseases.

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## Anti-HER2 immunoliposomes for targeted therapy of human tumors.

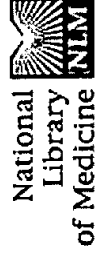
Park JW, Hong K, Kirpotin DB, Meyer O, Papahadjopoulos D, Benz CC.

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Anti-HER2 immunoliposomes (ILs) have been constructed by conjugation of Fab' fragments of recombinant humanized monoclonal antibody rhuMABHER2 to small sterically stabilized unilamellar liposomes, to create a targeted drug delivery vehicle for the treatment of HER2 (c-erbB-2, neu)-overexpressing cancers. Parameters affecting in vitro binding and internalization of ILs include liposome composition, Fab' linkage site and Fab' density. Anti-HER2 ILs have been constructed to optimize intracellular drug delivery. Doxorubicin (dox)-loaded ILs are highly stable and exhibit prolonged circulation in rats. In nude mice bearing HER2-overexpressing tumor xenografts, anti-HER2 ILs administered i.v. resulted in efficient tumor localization, with penetration of the ILs throughout the tumor mass and accumulation within tumor cells. In contrast, non-targeted liposomes resulted in extracellular tumor accumulation only. In multiple HER2-overexpressing human breast tumor xenograft models, treatment with dox-loaded anti-HER2 ILs produces significantly increased antitumor cytotoxicity as compared to free dox or dox-loaded non-targeted liposomes and significantly less systemic toxicity than free dox. To explore further the intracellular delivery advantages of ILs, anti-HER2 ILs bearing cationic lipids are being developed for nucleic acid delivery. These cationic immunoliposomes mediate efficient and specific transfection of target cells with reporter genes, as well as intracellular delivery of labeled oligonucleotides. Thus, anti-HER2 ILs represent an efficient and feasible strategy to achieve targeted intracellular delivery of therapeutic agents.

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## Noninvasive gene targeting to the brain.

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Gene therapy of the brain is hindered by the presence of the blood-brain barrier (BBB), which prevents the brain uptake of bloodborne gene formulations. Exogenous genes have been expressed in the brain after invasive routes of administration, such as craniotomy or intracarotid arterial infusion of noxious agents causing BBB disruption. The present studies describe the expression of an exogenous gene in brain after noninvasive i.v. administration of a 6- to 7-kb expression plasmid encoding either luciferase or beta-galactosidase packaged in the interior of neutral pegylated immunoliposomes. The latter are conjugated with the OX26 mAb to the rat transferrin receptor, which enables targeting of the plasmid DNA to the brain via the endogenous BBB transferrin receptor. Unlike cationic liposomes, this neutral liposome formulation is stable in blood and does not result in selective entrapment in the lung. Luciferase gene expression in the brain peaks at 48 h after a single i.v. administration of 10 microg of plasmid DNA per adult rat, a dose that is 30- to 100-fold lower than that used for gene expression in rodents with cationic liposomes. beta-Galactosidase histochemistry demonstrated gene expression throughout the central nervous system, including neurons, choroid plexus epithelium, and the brain microvasculature. In conclusion, widespread gene expression in the brain can be achieved by using a formulation that does not employ viruses or cationic liposomes, but instead uses endogenous receptor-mediated transport pathways at the BBB.

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